Remarks

Claims 42, 45, 26, and 51-68 were pending in the subject application. By this Amendment, claim 42 has been amended, claims 45, 46, 51, 60, 63, and 68 have been cancelled, and claims 69-78 have been added. The undersigned avers that no new matter is introduced by this amendment. Support for the new claims and amendments can be found throughout the subject specification and in the claims as originally filed. Entry and consideration of the amendments presented herein is respectfully requested. Accordingly, claims 42, 52-59, 61, 62, 64-67, 69-78 are currently before the Examiner for consideration. Favorable consideration of the pending claims is respectfully requested.

Applicants and Applicants' representative wish to thank Examiner Schnizer and Supervisory Examiner Schultz for the courtesy of the telephonic interview conducted with the undersigned on August 10, 2009, regarding the rejection under 35 USC §112, first paragraph, and 35 USC §103(a). The remarks and amendments set forth herein are consistent with the substance of the interview and are believed to address the outstanding issues as discussed during the interview.

By this Amendment, claim 42 has been amended and claims 69-78 have been added. Support for the amendments to claim 42 can be found, for example, at page 12, lines 12-24; page 14, lines 5-23 and 25; and page 15, lines 1-5 and 13-15 of the specification and the claims as filed. Support for claims 69, 70, 73-75 can be found, for example, in Examples 8-10 at pages 29-34 of the specification as filed. Support for claims 71 and 77 can be found, for example, at page 28, lines 24-28; page 30, lines 9-21; and page 31, lines 1-3 of the specification as filed. Support for claim 76 can be found, for example, at page 15, lines 1-6 and 24-26 of the specification as filed. Support for claim 78 can be found, for example, at page 10, lines 10-11, of the specification as filed. Support for claim 72 can be found, for example, at page 10, lines 1-14; page 12, lines 14-24; page 14, lines 5-23; page 21, lines 18 and 19; and Examples 7-10 at page 28-34 of the specification as filed.

Claims 42, 45, 46, and 51-63 are rejected under 35 USC §112, first paragraph, as non-enabled by the subject specification. The Examiner acknowledges that the specification enables methods of inhibiting expression of Dengue virus (DV) genes within a mouse by administering to the liver of the mouse a vector that expresses siRNA that reduces expression of a target DV gene by RNA interference. However, the Examiner asserts that the specification does not enable inhibition

of expression of DV genes in a human by any route of administration, or in an animal model by intramuscular, subcutaneous, intradermal, oral, or nasal administration of the vector. Applicants respectfully traverse and submit that the claimed invention is fully enabled by the subject specification.

As an initial matter, the Examiner asserts that, in view of the evidence of record, taken as a whole, those of skill in the art did not know the target cells for DV infection in humans at the time the application was filed, and concludes that one of skill in the art could not deliver the vector to the appropriate cells in a mammalian host to inhibit expression of DV genes without undue experimentation (pages 5, 7 and 8 of the Office Action). Submitted herewith for the Examiner's consideration are Libraty D.H. *et al.*, *Journal of Virology*, 2001, 75(8):3501-3508; Navarro-Sanchez E. *et al.*, *EMBO Reports*, 2003, 4(7):723-728; Lozach, P.Y. *et al., J. Biol. Chem.*, June 2005, 280(25):23698-23708, and Brandler, S. *et al., Am. J. Trop. Med. Hyg.*, January 2005, 72(1):74-81, which further support dendritic cells as target cells of DV (see abstract, page 3501, second column, and page 3507, last paragraph of Libraty D.H. *et al.*; abstract and page 723, second column, first full paragraph of Navarro-Sanchez E. *et al.*; abstract and page 23698, second column, second full paragraph of Lozach P.Y. *et al*;. and abstract and page 74, first column, third paragraph of Brandler S. *et al.*).

Furthermore, by this Amendment, independent claim 42 has been amended to recite that the vector is administered intravenously. As discussed during the telephonic interview, following the onset of illness, DV can be found in the blood and blood cells (see page 4472, first column, second paragraph of Wang W.K. *et al.*, *Journal of Clinical Microbiology*, 2002, 40(12):4472-4478; and page 642, second column, last full paragraph of the later published Shu P-Y *et al.*, *Clinical and Diagnostic Laboratory Immunology*, July 2004, 11(4):642-650, which are submitted herewith). Therefore, the cell type or types that are the initial target of DV is irrelevant to the effectiveness of the method of claim 42 as currently amended.

By this Amendment, Applicants have added independent claim 72, which recites a method for inhibiting Dengue virus (DV) infection and DV-induced apoptosis of human dendritic cells, comprising administering to the cells an effective amount of a vector comprising at least one gene suppressing cassette, wherein said gene suppressing cassette comprises a polynucleotide operably-

linked to a promoter sequence, wherein said polynucleotide encodes a short interfering RNA (siRNA) molecule that reduces expression of a target Dengue virus gene within the host by RNA interference, wherein the polynucleotide sequence is transcribed to produce the siRNA molecule. Based on the weight of the evidence of record, Applicants have shown that dendritic cells are a cellular target of DV. Assuming *arguendo* that dendritic cells residing in the skin are not the initial target cells of DV infection, the subject specification shows that blood dendritic cells are highly permissive to DV infection and susceptible to DV-induced apoptosis, and it is well established that the appearance of DV in the blood and blood cells coincides with presentation of symptoms (see Wang W.K. *et al.* (2002) and Shu P-Y *et al.* (2004), cited above). Therefore, in addition to the application of the method of claim 72 in the clinical context, Examples 8-10 of the specification make it clear to those skilled in the art that the claimed method is useful for the study of DV infection in this cell type and is enabled by the specification.

Next, the Examiner cites several publications pertaining to gene therapy as supporting the assertion that *in vivo* gene delivery and expression was "highly experimental" at the application's filing date and that delivery of the expression vector to the appropriate cells in a mammalian host to inhibit DV gene expression would require undue experimentation (Anderson *et al.*, 1998; Romano *et al.*, 2000; Rosenberg *et al.*, 2000). The single reference cited pertaining specifically to interfering RNA is Caplen *et al.* (2003). As indicated in the Office Action, Caplan *et al.* taught that "...the key issues of delivering nucleic acids to the required tissue and cell type, while ensuring an appropriate level of efficacy with minimum toxicity induced by the vector system..." (page 581). Applicants note, however, that the next sentence of the Caplan *et al.* publication states that "RNAi appears to have many advantages over that of the previous technologies developed for the downregulation of gene expression...". Additionally, the preceding paragraph of the Caplan *et al.* publication reports successful application of RNAi *in vivo* in mice.

Applicants submitted Milhavet O. *et al.*, *Pharmacol. Rev.*, December 2003, 55(4):629-648; Agrawal N. *et al.*, *Microbiol. Mol., Biol. Rev.*, December 2003, 67(4):657-685); Kim V.N. *et al.*, *J. Korean Med. Sci.*, 2003, 18:309-318; Gitlin L. and Andino, *J. Virol.*, 2003, 77(13):7159-7165; Coburn G.A. and Cullen, *J. Antimicrobial Chemotherapy*, 2003, 51:753-756; Lieberman J. *et al.*, *Trends Mol. Med.*, 2003, 9(9):397-403; Reich S.J. *et al.*, *Molecular Vision*, 2003, 9:210-216; Scherr

M. *et al.*, *Oligonucleotides*, 2003, 13:353-363; and Song E. *et al.*, *Nature Medicine*, 2003, 9(3):347-351 with the previous Response. Page 680 of the Agrawal N. *et al.* publication cites the therapeutic potential of siRNA *in vivo* in mouse models. The concluding remarks of Agrawal N. *et al.* indicate that "the knockdown technology would improve vastly with better-designed plasmid- or virus-based vectors for delivery of siRNAs to the appropriate tissues at the appropriate time." The Milhavet O. *et al.* publication reports the use of vector-driven shRNA delivery *in vivo* in a mouse model (Table 1). Applicants also submitted Tolentino M.J. *et al.* (*Retina*, 2004, 24:132-138). Reich S.J. *et al.* and Tolentino M.J. *et al.* demonstrate delivery of siRNA *in vivo* to reduce levels of targeted proteins in mice. For example, Tolentino M.J. *et al.* show a single intravitreal injection of vascular endothelial growth factor siRNA inhibited growth and vascular permeability of choroidal neovascularization in a non-human primate in a dose-dependent manner. The Examiner notes that this procedure involved local delivery of siRNA to the eye and indicates that, absent evidence that the eye comprises target cells for Dengue virus infection, the Tolentino *et al.* publication has no relevance. However, as indicated above, DV is found in the blood and independent claim 42 has been amended to recite that the vector is intravenously administered. Thus, the Tolentino *et al.* publication does support enablement of the claims as currently amended. Furthermore, submitted herewith is U.S. Patent Publication No. 20040018176 (Tolentino M.J. and Reich S.J.), which describes generation of vectors for siRNA delivery (Example 7) and includes working examples of *in vivo* delivery of siRNA to retinal cells of mice. Song E. *et al.* reports that intravenous injection of FAS siRNA reduced FAS mRNA levels and expression of FAS protein in mouse hepatocytes (see abstract). In addition, submitted herewith is the Zhang Y. *et al.* publication (*The Journal of Gene Medicine*, 2003, 5(12): 1039-1045), which demonstrates production and utilization *in vivo* of anti-luciferase shRNA expression plasmids encapsulated in pegylated immunoliposomes (PILs) and targeted across the blood-brain barrier and across the tumor cell membrane *in vivo* with a tethered monoclonal antibody to the rat transferring receptor. Intravenous administration of the shRNA expression plasmid to rats implanted with luciferase-expressing C6 rat glioma cells resulted in 90% inhibition of luciferase gene expression of in the brain cancer. Zhang Y *et al.* conclude:

> *In vivo* RNAi is enabled with a new form of gene delivery system that encapsulates expression plasmids in PILs, which are targeted to distant sites based on the

> specificity of a receptor-specific monoclonal antibody. The combined application of the PIL gene delivery system and RNAi expression plasmids enables gene silencing in remote sites such as brain cancer in mammals after intravenous administration (page 1039).

In view of the state of the art of RNAi at the time the subject application was filed, Applicants submit that the patent application contains sufficient disclosure to enable one of ordinary skill in the art to carry out the methods of the invention in humans without undue experimentation. Furthermore, in view of the disclosure of the subject specification as originally filed, which demonstrates that DV gene inhibition can be achieved and would be of benefit in inhibiting DV infection and DV-induced apoptosis of dendritic cells, the enablement provided by the specification is commensurate with the claimed methods as currently amended. Accordingly, reconsideration and withdrawal of the rejection under 35 USC §112, first paragraph, is respectfully requested.

Claims 42, 45, 46, 52, 53, 55, 58-60, 64, 65, and 68 are rejected under 35 USC §103(a) as obvious over Raviprakash *et al.* (*J. of Virology*, 1995, Vol. 69, pp. 69-74), An *et al.* (*Virology*, 1999, Vol. 263, pp. 70-77), Adelman *et al.* (*Journal of Virology*, 2002, Vol. 76, pp. 12925-12933), Tuschl *et al.* (U.S. Patent 7,056,704), Yu *et al.* (*PNAS*, 2002, Vol. 99, pp. 6047-6052), and Liu *et al.* (*Gene Therapy*, 1999, Vol. 6, pp. 1258-1266). In addition, claim 54 is rejected under 35 USC §103(a) as obvious over Raviprakash *et al.*, An *et al.*, Adelman *et al.*, Tuschl *et al.*, Yu *et al.*, and Liu *et al.* as applied to claims 42, 45, 46, 52, 53, 55, 58-60, 64, 65 and 68, and further in view of Adelman *et al.* (*Insect Mol. Biol.*, 2001, Vol. 10, pp. 265-273). Furthermore, claim 57 is rejected under 35 USC §103(a) as obvious over Raviprakash *et al.*, An *et al.*, Adelman *et al.* (2002), Tuschl *et al.*, Yu *et al.*, and Liu *et al.* as applied to claims 42, 45, 46, 52, 53, 55, 58-60, 64, 65 and 68, and further in view of Yu *et al.* (U.S. Patent 6,852,528). Claims 61 and 62 are rejected under 35 USC §103(a) as obvious over Raviprakash *et al.*, An *et al.*, Adelman *et al.*, Tuschl *et al.*, Yu *et al.* (2002), and Liu *et al.* as applied to claims 42, 45, 46, 52, 53, 55, 58-60, 64, 65 and 68, and further in view of Kumar *et al.* (U.S. Patent 7,067,633). Finally, claims 66 and 67 are rejected under 35 USC §103(a) as obvious over Raviprakash *et al.*, An *et al.*, Adelman *et al.*, Tuschl *et al.*, Yu *et al.* (2002), and Liu *et al.* as applied to claims 42, 45, 46, 52, 53, 55, 58-60, 64, 65 and 68, and further in view of Hope *et al.* (U.S. Patent 6,136,597). Applicants respectfully traverse.

The Examiner asserts that it would have been obvious to one of ordinary skill in the art at the time the application was filed to modify the method of Raviprakash *et al.* by substituting the antisense oligonucleotide with a vector encoding one or more siRNAs, and that one skilled in the art would have been motivated to do so in view of the teachings of Tuschl *et al.* Furthermore, the Examiner asserts that there would have been a reasonable expectation of success in reducing expression of the DV genes disclosed by Raviprakash *et al.* or Adelman *et al.* in the animal model of An *et al.*, using the method taught by Liu *et al.*, in view of the teachings of Yu *et al.* In reply, Applicants note that claim 51, which recites that the host is human, is excluded from the rejections under 35 USC §103(a). By this Amendment, Applicants have amended independent claim 42 to recite that the host is a human host, and cancelled claim 51. Applicants also note that claim 63, which recites that the vector is taken up by a dendritic cell, was also excluded from the rejections under 35 USC §103(a). New claim 72 recites a method for inhibiting Dengue virus (DV) infection and DV-induced apoptosis of human dendritic cells. As the Examiner appears to acquiesce, Applicants submit that the cited references do not provide one of ordinary skill in the art with a reasonable expectation of success in carrying out the claimed methods on a human host or human dendritic cells with any reasonable expectation of success.

The primary reference relied upon in each of the rejections under 35 USC §103(a) is the Raviprakash *et al.* publication, which describes experiments very different from those described in the working examples of the subject specification. The Raviprakash *et al.* publication discloses inhibition of DV genes *in vitro* in non-human (LLCMK/2 monkey kidney) cells using modified (propyne substituted) antisense oligonucleotides. Furthermore, the results of the Raviprakash *et al.* publication, independently or in combination with the other cited references, would not have lead one of ordinary skill in the art to the claimed methods with any reasonable expectation of success. The Raviprakash *et al.* publication discloses that unmodified antisense oligonucleotides were not effective in bringing about significant inhibition of DV (see abstract), and that the antisense oligonucleotide targeted against the 3' untranslated region (UTR) of the virus RNA showed "limited efficacy," attributing this latter result to the complex secondary structures presented by the large DV RNA (page 74, first full paragraph). In contrast to these results with 3' UTR-targeted antisense molecules, Applicants note that the subject specification demonstrates very effective inhibition of

DV infection and DV-induced apoptosis in human dendritic cells using a vector-mediated siRNA that targets a 3' UTR common to all four DV serotypes. These results are also presented and discussed in the Zhang W. *et al.* publication (*Genetic Vaccines and Therapy*, 2004, 2(8):1-10), which is co-authored by the inventors (see, for example, the abstract, Figures 3-5, and pages 6-10). Finally, the Raviprakash *et al.* publication concludes that the modified oligonucleotides may be generally more effective as antisense agents against other viruses (page 74, last sentence).

The An *et al.* publication is cited in the Office Action for teaching a mouse animal model for DV infection in which immunodeficient mice are transplanted with cells of a human hepatocarcinoma cell line (HepG2). Applicants note that the An *et al.* publication acknowledges that there is some question as to whether the described animal model is of any use in human gene therapy (see page 1265, first full paragraph). The Adelman *et al.* publication describes transformation of C6/36 mosquito cells with a plasmid designed to transcribe an inverted-repeat RNA from the DV genome, capable of forming double stranded RNA. The Yu *et al.* publication is cited for teaching RNA interference by expression of hairpin siRNAs and their use in mammalian cells. The Office Action concludes that one skilled in the art would have been motivated to substitute the antisense oligonucleotides of the Raviprakash *et al.* publication because the Tuschl *et al.* publication taught that siRNAs were more efficient than antisense.

Obviousness does not require absolute predictability; however, at least some degree of predictability is required. MPEP §2143.02. Evidence showing there was no reasonable expectation of success may support a conclusion of nonobviousness. *In re Rinehart*, 531 F.2d 1048, 189 USPQ 143 (CCPA 1976). Furthermore, assuming *arguendo* that it would have been obvious to try administering the claimed vector to the mouse model of An *et al.* in view of the cited references at the time the application was filed, this would not render obvious the claimed methods reciting administration to a human host or human dendritic cells. It is well established that obvious to try is an acceptable rationale in support of a conclusion of obviousness when choosing from a finite number of identified, predictable solutions, with a reasonable expectation of success. MPEP §2141. Such is not the case here.

At pages 13 and 14 of the Office Action, the Examiner asserts that it would have been obvious to administer the siRNA vector either prior to, or after, DV infection. Applicants submit

that success in reducing DV gene expression in a host by administering siRNA post-infection does not necessarily provide a reasonable expectation that prophylactic administration would be effective in the absence of empirical evidence.

Finally, Applicants submit that the cited references provide no reasonable expectation that administration of the claimed vector would be effective in inhibiting DV infection and DV-induced apoptosis of human dendritic cells without triggering an acute inflammatory response, as demonstrated in Examples 8-10 of the specification and Figures 3-5 of Zhang W. *et al.* (2004). As indicated in the Zhang W. *et al.* publication, attenuation of DV infection in dendritic cells and protection of infected dendritic cells would be a benefit for the elimination of the early DV infection and the development and maintenance of antiviral innate/adaptive immune response *in vivo* (see penultimate paragraph at page 10 of Zhang W. *et al.*).

Applicants respectfully submit that the claimed invention is not obvious over the cited references. Accordingly, reconsideration and withdrawal of the rejections under 35 USC §103(a) is respectfully requested.

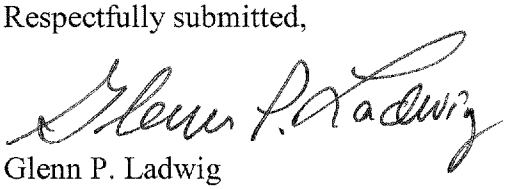
It should be understood that the amendments presented herein have been made solely to expedite prosecution of the subject application to completion and should not be construed as an indication of Applicants' agreement with or acquiescence in the Examiner's position.

In view of the foregoing remarks and amendments to the claims, Applicants believe that the currently pending claims are in condition for allowance, and such action is respectfully requested.

The Commissioner is hereby authorized to charge any fees under 37 CFR §§1.16 or 1.17 as required by this paper to Deposit Account 19-0065.

Applicants invite the Examiner to call the undersigned if clarification is needed on any of this response, or if the Examiner believes a telephonic interview would expedite the prosecution of the subject application to completion.

Respectfully submitted,



Glenn P. Ladwig
Patent Attorney
Registration No. 46,853
Phone No.: 352-375-8100
Fax No.: 352-372-5800
Address: P.O. Box 142950
Gainesville, FL 32614-2950

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Attachment:
Libraty D.H. *et al.*, *Journal of Virology*, 2001, 75(8):3501-3508
Navarro-Sanchez E. *et al.*, *EMBO Reports*, 2003, 4(7):723-728;
Lozach, P.Y. *et al., J. Biol. Chem.*, June 2005, 280(25):23698-23708
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Zhang, Y. *et al., The Journal of Gene Medicine*, 2003, 5(12):1039-1045
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